Journal of Advances in Microbiology

6(2): 1-18, 2017; Article no.JAMB.37259
ISSN: 2456-7116

Infective Keratitis in Alexandria Main University Hospital

Zainab Abdelkader[1*], Alaa Ghaith[2], Soraya El- Shazy[1] and Walaa Hazzah[1]

[1]Department of Microbiology, High Institute of Public Health (HIPH), Alexandria University, Egypt.
[2]Department of Ophthalmology, Faculty of Medicine, Alexandria University, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author ZA designed the study and protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors WH and AG managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2017/37259
Editor(s):
(1) Foluso O. Osunsanmi, Department of Biochemistry and Microbiology, University of Zululand, South Africa.
Reviewers:
(1) Engy M. Mostafa, Sohag University, Egypt.
(2) Asaad Ahmed Ghanem, Mansoura University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/21528

Original Research Article

Received 9th October 2017
Accepted 18th October 2017
Published 24th October 2017

ABSTRACT

Background: Blindness is and, apparently always has been, a problem in Egypt. Corneal blindness is a major public health problem in which; 1.5–2.0 million new cases of monocular blindness reported annually in developing countries is secondary to corneal ulceration. Bacterial keratitis is one of the most threatening ocular infectious pathologies that can lead to severe visual disability. To help avoiding the specific therapy risks of disease progression and the microbiological investigations being incomplete or misleading, other organisms as virus, fungi, and *Acanthamoeba* should be considered.
Aims: To isolate and identify different bacterial agents causing keratitis and identify factors associated with bacterial keratitis.
Study Design: This cross-sectional study was carried out to identify causative pathogens and to determine the demographic characteristics, predisposing factors of keratitis (corneal ulcer).
Place and Duration of Study: Sample: Department of Microbiology, in High Institute of Public Health, and Department of Ophthalmology, Faculty of Medicine, Alexandria University, between; August, 2014 to May, 2015.
Methodology: A total of 100 cases were examined, samples (corneal swab and scrapings) were collected from clinically diagnosed corneal ulcer patients attending Ophthalmology outpatient clinic

**Corresponding author: E-mail: zainab_badawy@hotmail.com;*

of Alexandria Main University Hospital. Samples were processed by corneal smear microscopy (potassium hydroxide and Gram stains) and culture examination (5% sheep blood agar, sheep blood chocolate agar, and Sabouraud dextrose agar and brain heart infusion).
Results: Out of 100 cases, 49 (52.1%) cases had bacterial growth, 32 (34%) patients showed fungal growth, 20 (21.3%) cases had viral keratitis and 24 (25.5%) cases had *Acanthamoeba* corneal infestation. The predominant bacterial isolates were *Staphylococcus epidermidis* 24 (48%) followed by *Pseudomonas species* 8 (16%). *Aspergillus species* 16 (50%) were the most common fungal isolates followed by *Fusarium species* 10 (31.2%). Common associated factors were diabetes mellitus (29%), and corneal trauma (17%).
Conclusions: Diabetes was the most common general risk factor while corneal trauma was the most common local cause. The main causative organism of microbial keratitis was bacteria, where *Staphylococcus spp.* the main agent followed by *P. aeruginosa*. Vancomycin and fluoroquinolones showed higher rates of sensitivities on bacteria compared to other antibacterial agents.

Keywords: Corneal ulcer; infective keratitis; bacterial infection; health informatics.

1. INTRODUCTION

Microbial keratitis is a potentially serious corneal infection and a major cause of visual impairment worldwide [1]. It is caused by various organisms, such as bacteria, fungi, viruses, or protozoa. A conservative estimate of the number of corneal ulcers occurring annually in the developing world alone is 1.5–2 million [2]. The severity of corneal infections usually depends on the underlying condition of the cornea and the virulence of the infecting microbes [3].

Microbial corneal ulcers need a complete laboratory work up owing to considerable overlap in the clinical appearance of corneal ulcers due to various microorganisms [4]. In the era of modern medical technology, various methods of investigations are available for corneal ulcers including; microbial smears and cultures, antibiotic sensitivity, corneal biopsy and polymerase chain reactions (PCR). Microbiological investigations are easily performed, less invasive and cost-effective and also provide prompt diagnosis hence they are considered gold standard investigations. There are various approaches to the microbiological investigation of patients with suspected keratitis. Traditional methods include the use of multiple corneal scrapes with direct inoculation onto different enrichment media. For example, multiple studies used sheep's blood agar (BA), chocolate agar (CA), non-nutrient, Sabouraud agar, and brain–heart infusion broth (BHIB). Apart from the diagnostic value of corneal scraping, it may accelerate disease resolution by enhancing antibiotic penetration and therapeutic debridement of necrotic tissue [5,6].

Thus, the identification of the specific causative organism aids in incorporation of the most specific treatment drug which reduces the irrational use of multiple medications, the ocular toxicity and the emergence of resistance [7]. Hence, the present prospective study was undertaken to determine the epidemiological features, predisposing factors and clinical presentations of microbial keratitis and the efficacy of commonly used topical antibiotics.

It is very important for the best prognosis in keratitis cases, to confirm the clinical diagnosis by the laboratory work since the main aim is to start immediately the specific medical treatment. The laboratory support in the clinical diagnosis of keratitis is very important in order to achieve a shorter evolution time and to achieve a small scar for the better visual acuity in a patient suffering for a corneal infection.

The time lag between the clinical assessment and the laboratory investigation reveals the importance of accurate clinical diagnosis. As the cornea is considered a critical organ in which the patient must start medication before the lab results show up. The accurate clinical diagnosis needs an expert ophthalmologist to be able to diagnose the infectious keratitis causes and gives the needed treatment or at least the nearest measures. The decision support systems and information technologies can aid the ophthalmologist in assessment and ensure its' accuracy. Clinical decision support system (CDSS) aims to prevent medical errors through changing the way of practicing medicine [8]. They also provide clinicians, staff, patients, and other individuals with knowledge and person-specific information, intelligently filtered and

presented at appropriate times, to enhance health and health care [9].

2. METHODOLOGY

This is a cross sectional study conducted at the High Institute of Public Health (HIPH) microbiology lab and the Ophthalmology outpatient clinic of Alexandria Main University Hospital. It was to estimate cases with presumed infective microbial keratitis recently admitted to the hospital between August 2014 and May 2015. This study was approved by the Ethics Committee at Institute of Public Health (HIPH), Alexandria University.

2.1 Collection and Identification of Microbial keratitis

Hundred patients were examined and data was collected in relation to the demographic features, risk factors including use of topical steroids, trauma, ocular surface diseases, contact lens usage, therapeutic regimens received and systematic diseases as diabetes.

2.2 Corneal Sampling

Microbial keratitis was defined and corneal scraping was performed under topical anaesthesia following a standard protocol. Corneal specimens were collected using Kimura spatula under slit lamp.

2.3 Culture and Identification Procedures

The scraped materials were inoculated into a liquid medium (BHIB) [10,11] and directly streaked on culture plates (fresh Blood agar, Chocolate agar & Sabouraud dextrose agar media) in a “C-streaked” pattern to localize the site of implantation [12,13]. Strict asepsis was observed during the sample processing and their transport to the laboratory. Blood agar plates were incubated under aerobic condition, chocolate agar plates were incubated in 5-10% carbon dioxide both were evaluated at 24 hours and 48 hours and then discarded if no growth was seen [14]. SDA plates with chloramphenicol were incubated at 25°C for 5-7 days.

The inoculated BHIB was incubated for 24hrs at 37°C and a smear was prepared from this liquid medium for gram staining, in addition, BA, CA, and SDA plates were subculture and incubated as described previously [12,13].

Isolation and identification of bacterial and fungal spp. were done as per standard guidelines as well as the sensitivity results [15]. Bacterial and fungal isolates were also confirmed using the MALDI-TOF MS device (ultrafleXtreme BRUKER device) in Alexandria Main University Hospital. In cases that were nonresponsive to treatment and clinically suspicious of *Acanthamoeba*, smear was done for chromotrope staining procedure. The viral keratitis cases were identified clinically.

2.4 Statistical Analysis of the Data

Data were analysed using Statistical Package for Social Sciences software package version 18.0 (SPSS, Chicago, IL, USA). Qualitative data was expressed in frequency and per cent. Qualitative data was analysed using Fisher’s exact test and Monte Carlo was applied to compare different groups. P-value was assumed to be significant at 0.05.

2.5 Data Mining

Systematic approaches were assembled using pre-specified certain risk factors and diagnostic signs, which resulted in extraction of information depending on these variables to build up functional software package data mining. The Apriori algorithm is used to perform association analysis on the attributes of patient risk factors, clinical features and microbiological diagnosis.

3. RESULTS AND DISCUSSION

3.1 Epidemiological Characteristics

In our study, the majority of patients were males 67% (67/100) and females were only 33% (33/100) out of 100 patients. The most susceptible age group to keratitis was adults of age 21-60 years (76%, 76/100) with the highest number of cases being recorded among patients of 41-60 years (43% of the total cases). The least percentage fall in the age group <20 (5%, 5/100), most of them were females (80%, 4/5), whereas the elderly group >60 (19%, 19/100) were mostly men representing (89.5% of elderly patients, 17/19), and the average age of presentation was 46.7 years. (Fig. 1) The majority of the patients were from rural population (63%); compared to 37% patients were from urban population. The monthly incidence of cases varies throughout the 10 months period, where the highest number of cases (40%) was recorded in August-to-October period of time, followed by March-to-May (33%),

whereas, the least number of patients (27%) were admitted in the winter season (November-February). Out of 100 patients enrolled in this study, a large number of patients were farmers (42%, 42/100), all were males. However, the majority of females were domestic workers (11% 11/100). Laborers and other industrial workers were 30%, (30/100).

3.2 Clinical Presentations

Majority of patients in this study presented during the first two weeks of the onset of symptoms (74%), specifically during the first week (39%), while 16% of subjects presented during 15-30 days and the remaining 10% presented after 30 days since onset of symptoms. The symptoms of microbial keratitis are similar in most patients. The severity may vary in relation to the underlying causative organism, the immune status of the host, and the duration of the symptoms before presentation. In this study, all patients presented with pain, redness, photophobia and reduction in vision. The right eyes were involved in 63.5% of the patients. The ulcer shape varied between nonspecific (69%), geographic (16%) and serrated (15%). Centrally located ulcers were more common (56%) while, 32% of cases showed peripherally located ulcer.

3.3 Risk Factors

The most common risk factor in this study was diabetes (29 cases) as a systematic cause followed by smoking as a bad habit in 21 cases, while the most common local causes were corneal trauma, past-ocular disease/surgery and contact lens usage showing same percentage among keratitis patients (17 cases each). Topical steroid intake was present in 12% of the patients, and 13% had history of hypertension disease. Nevertheless, cases showing no predisposing factor accounted 19%, whereas, 39% patients suffered from multiple risk factors (Fig. 2).

3.4 Microbial Investigations

The prevalence of microbial keratitis was 94% among these 60% of the cases were suffering from single microbe as follow; bacterial (29%), fungal (3%), *Acanthamoeba* (12%) and viral keratitis (16%) of total cases. Moreover, poly-microbial keratitis were present in 34 cases, the higher combination was bacterial-fungal keratitis (15 cases), whereas, viral keratitis equally superadded by fungal and bacterial organisms (2 cases each). However, *Acanthamoeba* was usually superadded by fungal keratitis (10/24 cases), and to lesser extent mixed with bacteria (2/24 cases). The indefinite diagnosis represented 6 cases in which they received anti-inflammatory drugs with prophylactic antibiotics.

The frequency of isolation of bacteria in corneal scrapings were 32 (64%) Gram positive bacterial isolates, and 18 (36%) were Gram negative. Among Gram positive bacteria, the most commonly isolated organism was *S. epidermidis* 24 (48%), followed by *S. aureus* (12%), while,

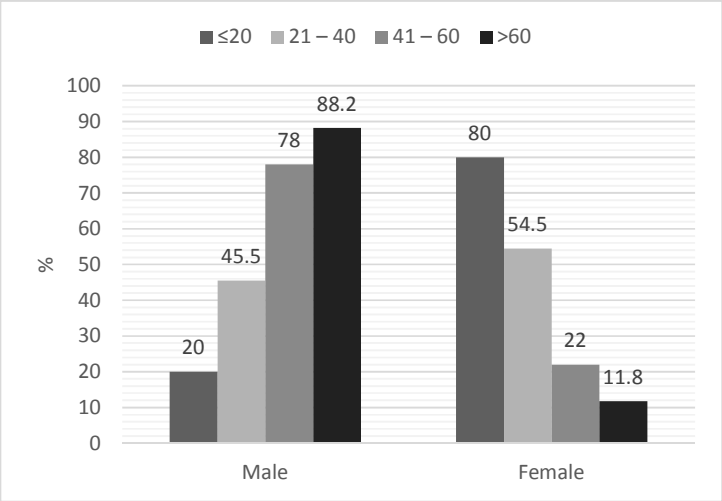

Fig. 1. Distribution of 100 studied microbial keratitis patient's age in relation to gender

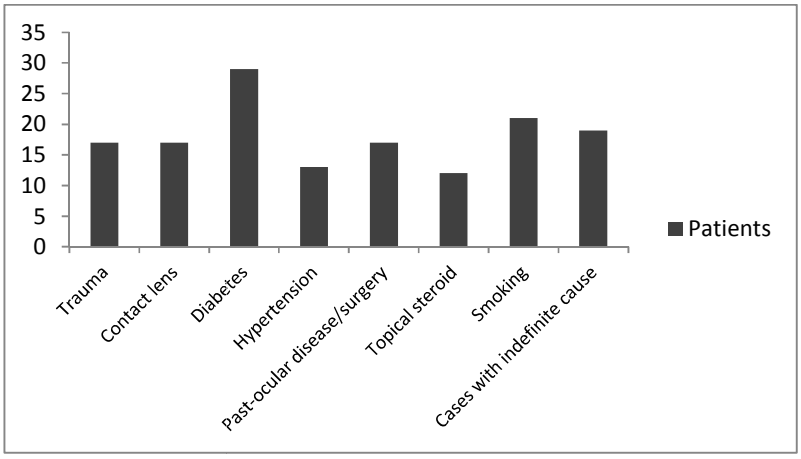

Fig. 2. Factors studied in 100 patients with suspected microbial keratitis

among Gram negative bacteria, the most commonly isolated organism was *P. aeruginosa* (16%), followed by *K. pneumoniae* (12%). The majority of patients (50%) with fungal keratitis had *Aspergillus* spp., followed by 10 isolates (31.2%) *fusarium* spp., while *candida* spp. were found in 6 cases (18.8%).

3.5 Data Mining Analysis

The data mining techniques showed great correlation between various causative organisms of keratitis and clinical findings beside other predisposing factors. Based on this research, using Apriori algorithm on infectious keratitis dataset, the confidence was ranging between 100% and 97%. Moreover, there was a relation between topical steroid application, satellite lesions and fungal infection, whereas, there was an association between contact lens usage, perineuritis clinical finding in the affected cornea and *Acanthamoeba* keratitis. As for bacterial keratitis; by applying the Decision Tree (J48) single-label classifier; the F-score was 97% and the accuracy 97%; it showed that when the onset is rapid it is most probably bacterial element. In addition, there was a high prevalence of recurrence in relation with viral infection (Fig. 3).

3.6 Media Variation

The bacterial and fungal isolates recovered by both liquid (BHIB) and solid media (BA, CA, SDA) revealed 66.7% positive cases, where 36.5% appeared in liquid media only while 30.2% were positive in both solid and liquid media.

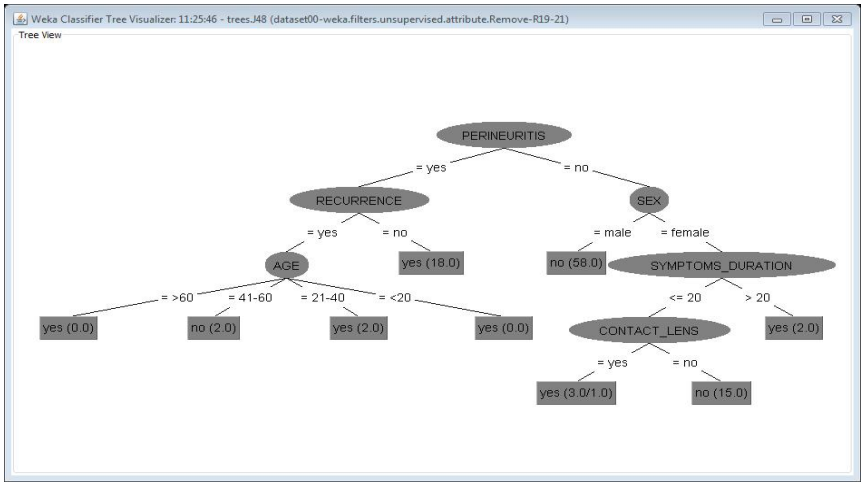

Fig. 3. *Acanthamoeba* keratitis decision tree (J48)

3.7 Antimicrobial Susceptibility Pattern of Bacterial Isolates

The antimicrobial susceptibility pattern of bacterial isolates of the most commonly used antibiotics was as follow; *S. aureus* was highly sensitive to vancomycin and amikacin (100% each), followed by neomycin and clindamycin (83.3% each), and 66.7% sensitivity to macrolids, flouroquinolones, however, 100% were resistant to third generation cephalosporin. Compared to *S. epidermidis,* isolates showed less sensitivity to vancomycin, amikacin, and gentamycin (79.2% each), followed by lower response to macrolids and flouroquinolones except for moxifloxacin (87.5%).

On the other hand, *E.coli and K. pneumoniae* were 100% sensitive to most antibacterial tested, except for macrolides and clindamycin. While the resistance rate for *Pseudomonas* spp. to antibiotics like neomycin, cefoxitin, macrolides and tetracyclin was more than 50%.

Chloramphenicol, the frequently used ophthalmic antibiotic was found to be effective for most of the isolates except for *S. aureus* and *P. aeruginosa* which showed 83% and 87.5% resistance, respectively. On contrary of flouroquinolones, all Gram negative bacteria were sensitive to this group while Gram positive bacteria were less sensitive (Table 1a-b).

Table 1a. The antimicrobial susceptibility of isolated Gram positive bacteria

	***S. aureus* (n = 6)**		***S. epidermidis* (n = 24)**		***Bacillus spp.* (n = 1)**		***Corynebacterium spp.* (n = 1)**	
Antibiotics	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Penicillin								
Oxacillin	2	33.3	12	50%	1	100%	1	100%
Cephalosporin								
Cefoxitin 2nd	2	33.3%	14	58.3%	1	100%	0	0%
Ceftriaxone 3rd	0	0%	11	45.8%	1	100%	0	0%
Cefotaxime 3rd	0	0%	9	37.5%	1	100%	0	0%
Ceftazidime 3rd	0	0%	3	12.5%	1	100%	0	0%
Glycopeptide								
Vancomycin	6	100%	19	79.2%	1	100%	1	100%
Aminoglycoside								
Gentamycin	4	66.7%	19	79.2%	1	100%	0	0%
Tobramycin	4	66.7%	10	41.7%	1	100%	0	0%
Amikacin	6	100%	19	79.2%	1	100%	0	0%
Neomycin	5	83.3%	10	41.7%	1	100%	0	0%
Fluoroquinolones								
Ciprofloxacin 2nd	4	66.7%	13	54.2%	1	100%	1	100%
Ofloxacin 2nd	4	66.7%	11	45.8%	1	100%	1	100%
Norfoxacin 2nd	4	66.7%	13	54.2%	1	100%	0	0%
Lomefoxacin 2nd	4	66.7%	13	54.2%	1	100%	1	100%
Levofloxacin 3rd	4	66.7%	15	62.5	1	100%	1	100%
Moxifloxacin 4th	4	66.7%	21	87.5%	1	100%	1	100%
Macrolide								
Erythromycin	4	66.7%	10	41.7%	1	100%	0	0%
Azithromycin	4	66.7%	8	33.3%	1	100%	0	0%
Clarithromycin	4	66.7%	10	41.7%	1	100%	0	0%
Tetracyclin								
Doxycyclin	4	66.7%	10	41.7%	1	100%	1	100%
Tetracyclin	0	0%	8	33.3%	0	0%	0	0%
Bacteriostatic								
Fusidic acid	0	0%	14	58.3%	1	100%	1	100%
Chloramphenicol	1	16.7%	17	70.8%	1	100%	1	100%
Polymyxin B	0	0%	8	33.3%	0	0%	0	0%
Clindamycin	5	83.3%	16	66.7%	1	100%	0	0%

Table 1b. The antimicrobial susceptibility of isolated Gram negative bacteria

	E. coli (n = 2)		*K. pneumoniae* (n = 6)		*P. aeruginosa* (n = 8)		*S. marcescens* (n = 2)	
Antibiotics	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Penicillin								
Oxacillin	0	0%	2	33.3%	0	0%	0	0%
Cephalosporin								
Cefoxitin 2nd	2	100%	6	100%	0	0%	1	50%
Ceftriaxone 3rd	2	100%	6	100%	3	37.5%	2	100%
Cefotaxime 3rd	2	100%	6	100%	6	75%	2	100%
Ceftazidime 3rd	0	0%	6	100%	6	75%	2	100%
Aminoglycoside								
Gentamycin	2	100%	6	100%	7	87.5%	2	100%
Tobramycin	2	100%	6	100%	8	100%	1	50%
Amikacin	2	100%	6	100%	8	100%	2	100%
Neomycin	2	100%	6	100%	1	12.5%	1	50%
Fluoroquinolones								
Ciprofloxacin 2nd	2	100%	6	100%	8	100%	2	100%
Ofloxacin 2nd	2	100%	6	100%	8	100%	2	100%
Norfoxacin 2nd	2	100%	6	100%	8	100%	2	100%
Lomefoxacin 2nd	2	100%	6	100%	8	100%	2	100%
Levofloxacin 3rd	1	50%	6	100%	8	100%	2	100%
Moxifloxacin 4th	2	100%	6	100%	8	100%	2	100%
Macrolide								
Erythromycin	0	0%	0	0%	1	12.5%	0	0%
Azithromycin	0	0%	0	0%	1	12.5%	0	0%
Clarithromycin	0	0%	0	0%	1	12.5%	0	0%
Tetracyclin								
Doxycyclin	0	0%	6	100%	1	12.5%	0	0%
Tetracyclin	0	0%	6	100%	1	12.5%	0	0%
Bacteriostatic								
Chloramphenicol	2	100%	6	100%	1	12.5%	2	100%
Polymyxin B	0	0%	2	33.3%	8	100%	0	0%
Clindamycin	0	0%	0	0%	1	12.5%	0	0%

4. DISCUSION

4.1 Microbial Keratitis Trend and Presentations

Bacterial keratitis is an ophthalmic emergency that needs immediate treatment. Our study focuses on the pattern of bacterial pathogen causing keratitis and the antibiogram of the bacterial isolates among patients attending the outpatient clinic in the Ophthalmology, Alexandria Main University Hospital.

The majority of patients presented during first two weeks of onset of the symptoms (74%), specifically during the first week (39%), similar to the study of Saka*,* et al. [16] *where* (79.6%) cases presented during first two weeks.

Centrally located ulcers were more common (56%) while, 32% of cases showed peripherally located ulcer. A study in Jordan [17] found 60% of microbial keratitis affecting central two-thirds of the cornea, compared to 14.8% reported in Saudi Arabia [18].

4.2 Data Mining Techniques Interpretations

The data mining techniques showed great correlation between various causative organisms of keratitis and clinical findings beside other predisposing factors. For example, by applying data mining decision tree, it revealed that the rapid onset/course of the disease appeared to have a great correlation with bacterial keratitis, and *Acanthamoeba* keratitis was more likely to occur in younger aged group, and in patients with a longer duration of symptoms. The average range of patients' age with *Acanthamoeba* keratitis in this study (20-60 years) was similar to a study in New Zealand [19], which could be due

to that younger aged patient might be using contact lenses. Furthermore, other clinical findings including ring infiltrate/immune ring (25%) and perineuritis (22%) appeared to be highly associated with *Acanthamoeba* keratitis. It is possible that the immune ring is an indicator of prolonged untreated infections, which would be consistent with the longer duration of symptoms in the *Acanthamoeba* group of this and other studies [20,21]. As regard patients complaining of satellite lesions (30%), the majority of them were suffering from fungal keratitis and that was proven by previous study [22]. About 18% of cases had history of recurrent corneal lesions. This is higher than the findings of Geilani, et al. [23] where only 4.5% had history of recurrent keratitis. Recurrence of keratitis symptoms did not emerge as an important risk factor, though it is predominant associated with viral keratitis (15 of 18 viral cases) which was proved by data mining techniques, and was observed in a previous study [24].

4.3 Risk Factors In Relation to Microbial Findings

Out of 100 cases, the most common risk factor was diabetes (29%) followed by smoking in 21% of the cases, while the most common local causes were corneal trauma, past-ocular disease/surgery and contact lens usage (17 cases each). Prokosch et al. [25] showed similar results, as diabetes was the most common systematic predisposing factor and regarding local factors contact lens usage was predominant. Keay, et al. [26] stated that diabetes and smoking had great role as being systematic causes for microbial keratitis. However, multiple studies [14,16] proved that trauma as a local cause was the most dominant risk factor, which did not appear obviously in this study.

Corneal trauma and steroid usage were the major predisposing factors to fungal keratitis. The correlation between trauma and fungal keratitis was highly significant in a study of 3183 patients at Tamil nadu, [13] and that was also stated in previous studies [27,28].

In our study, despite that contact lens usage did not emerge as an important risk factor, it was considered as the major predisposing factor for *Acanthamoeba* (12/24) cases, and it is a predominant risk factor for *Acanthamoeba* keratitis in developed countries as well [29].

4.4 Microbial Findings

Bacterial organisms had the higher incidence (50%) causing microbial keratitis, followed by fungi (32%), *Acanthamoeba* (24%), and viruses (20%). In Egypt, multiple studies on microbial keratitis were carried revealing the following results; bacterial keratitis (55%) [14], (56.7%) [30], fungal infection (35%) [14], (18.7) [30], *Acanthamoeba* keratitis (19.1%) [31], and viral keratitis were positive by cell culture in (20.8%), whereas PCR was positive in (29.2%) cases. A study conducted in China showed that percentages of bacterial, fungal, *Acanthamoeba*, viral keratitis were 46.2%, 10.2%, 0%, and 43.6% respectively [32]. In addition, a study carried out in the United Kingdom (UK) found the percentages of microbial corneal ulcer as follow; bacteria (58.2%), fungal (3%), *Acanthamoeba* (0.5%) and viral infection (19.9%) [33]. However, most of the studies did not encounter viruses with other infective agents and considered the microbial keratitis as bacterial, fungal and *Acanthamoeba* keratitis only.

4.4.1 Bacterial isolates

Gram positive organisms (64%) represented the preponderance, (63.5%), where *Staphylococcus* spp. were the most common agents in 60% of isolates, which is consistent with results from Gopinathan, et al. [34] MRSA was present in 66.7% of the *S. aureus* isolates, whereas, MRSE was present in 41.7% of the *S. epidermidis* isolates. Lichtinger, et al. [35] conducted a 11-year review study and stated that there was a trend toward increasing laboratory resistance to methicillin from 28% during the first 4 years of the study to 38.8% for the last 3 years.

P. aeruginosa was the predominant Gram-negative bacteria accounting for 16% of the total bacterial isolates, which was in agreement with the results of Tewari, et al. [36] followed by *K. pneumoniae* (12%) *E. coli* (4%) and *Serratia marcescens* (4%).

4.4.2 Fungal isolates

Aspergillus species were the most common isolated fungi (50%), followed by *Fusarium* spp. (31.2%) and *Candida* spp. (18.8%). In agreement with the current results, Al-Hussaini, et al. [14] reported that *Aspergillus* spp., *Fusarium* spp., and *Candida* spp. accounted for 60%, 10% and 6%, respectively of total fungal isolates.

4.5 Solid and Liquid Media Variation

Indirect culture method increased the chance of isolation of bacteria and fungi in pure and /or mixed infections. The isolated organisms were 34 bacteria, 15 fungal and 15 mixed, in which 29-single isolates were recovered by direct culture compared to the indirect culture, using BHIB, with 64 cases of single or mixed positive growth. Bhadange, et al. [37] stated that liquid culture media had great role in isolation of bacterial organisms and mixed infections.

4.6 Antibiotic Susceptibility of Bacterial Isolates Interpretation

S. aureus showed highest sensitivity to vancomycin and amikacin, whereas, the most resistance was to the third generation cephalosporins (ceftriaxone, cefotaxime and ceftazidime), tetracycline, fusidic acid and polymyxin B. The *S. epidermidis* were mainly sensitive to the fourth generation fluoroquinolones (moxifloxacin), while the high resistance was counted to ceftazidime. The most effective antibiotics against Gram negative bacteria were aminoglycosides (tobramycin, amikacin mainly) and fluoroquinolones. In conclusion, fluoroquinolones had the highest susceptibility against all isolated bacteria (62%).

In conclusion, proper handling of contact lenses, topical steroids and antimicrobials is needed to evade predisposing factors of microbial keratitis. The use of combined direct and indirect culture methods is recommended for better recovery of microorganisms in suspected bacterial and fungal keratitis. Vancomycin, aminoglycosides and fluoroquinolones are the best choices for shotgun (initial) therapy of suspected bacterial keratitis. Finally, further studies using data mining techniques are required to help introducing decision support systems and information technologies in the healthcare systems.

5. CONCLUSIONS

The present study provides that corneal trauma is a major local cause, while diabetes is considered to be a major general risk factor for corneal infection. In addition, bacterial organisms are thought to be the main causative organism of microbial keratitis, where *Staphylococcus* spp. the main agent followed by *P. aeruginosa*. According to the antimicrobial susceptibility, Vancomycin and fluoroquinolones showed higher rates of sensitivities against different bacterial isolates in comparison to other antibacterial agents. This study, applied a comparison between liquid and solid media in relation to the ability to recover bacterial/ fungal organisms in single and/ or mixed infection, in which, liquid media increased the chance of isolation of bacteria and fungi in pure and /or mixed infection. It was noticed that using - MALDI-TOF, would simplify the process of microbial identification in terms of accuracy and rapidity.

CONSENT

All authors declare that 'written informed consent was obtained from participants (or other approved parties).

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee "High Institute of Public Health Ethical Committee, Alexandria University, Egypt, and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

ACKNOWLEDGEMENT

Prof. Dr. Osama Mohammed Badawy, Professor of Computer Science, Arab Academy for Science and Technology, Alexandria, Egypt, who helped in the "Data Mining" section.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shah A, et al. Geographic variations in microbial keratitis. Br J Ophthalmol. 2011; 95(6):762-7.
2. Whitcher JP, Srinivasan M, Upadhyay MP. Corneal blindness: A global perspective. Bull World Health Organ. 2001;79(3):214-21.
3. Ahn M, et al. Clinical aspects and prognosis of mixed microbial (bacterial and fungal) keratitis. Cornea. 2011;30(4):409-13.

4. Allan BD, Dart JK. Strategies for the management of microbial keratitis. Br J Ophthalmol. 1995;79(8):777-86.
5. Foster CS, Azar DT, Dohlman CH. *S*molin and thoft's cornea. 4th ed. New York: Williams and Wilkins; 2005.
6. Arffa RC. *G*rayson's Diseases of the cornea. 4th ed. St. Louis: Mosby; 1997.
7. Garg P. Investigative modalities in infectious keratitis. Indian J Ophthalmol. 2009;57(2):159.
8. McCartney PR. Clinical decision support systems. MCN Am J Matern Child Nurs. 200732(1):58.
9. Osheroff JA, et al. A roadmap for national action on clinical decision support. J Am Med Inform Assoc. 2007;14(2):141-5.
10. Amatya R, et al. Etiological agents of corneal ulcer. Nepal Med Coll J. 2012; 14(3):219-22.
11. Schaefer F, et al. Bacterial keratitis: A prospective clinical and microbiological study. Br J Ophthalmol. 2001;85(7):842-7.
12. Srinivasan M, et al. Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, south India. Br J Ophthalmol. 1997;81(11):965-71.
13. Bharathi MJ, et al. Epidemiological characteristics and laboratory diagnosis of fungal keratitis. Indian J Ophthalmol. 2003; 51(4):315-21.
14. Al-Hussaini AK, et al. Human microbial keratitis in upper Egypt. J Basic Appl Mycol Egypt. 2010;1:1-10.
15. Forbes B, Sahm D, Weissfeld A. Bailey and scott's diagnostic microbiology. 12th ed. St louis: Mosby; 2007.
16. Saka SE, et al. *P*resentation and outcome of microbial keratitis in Ilorin, Nigeria. British Journal of Medicine and Medical Research. 2015;6(8):795-803.
17. Bataineh H, Hammory Q, Khatatba A. Bacterial Keratitis: Risk Factors and causative agents. Sudan Medical Journal. 2008;3.
18. Aldebasi YH, et al. *I*ncidence and risk factors of bacteria causing infectious keratitis. Saudi Med J. 2013;34(11):1156-60.
19. Patel DV, Rayner S, McGhee CN. Resurgence of Acanthamoeba keratitis in Auckland, New Zealand: A 7-year review of presentation and outcomes. Clin Experiment Ophthalmol. 2010;38(1):15-20; quiz 87.
20. Tu EY, et al. Prognostic factors affecting visual outcome in Acanthamoeba keratitis. Ophthalmology. 2008;115(11):1998-2003.
21. Por YM, et al. Acanthamoeba keratitis associated with contact lens wear in Singapore. Am J Ophthalmol. 2009;148(1): 7-12e2.
22. Dalmon C, et al. The clinical differentiation of bacterial and fungal keratitis: a photographic survey. Invest Ophthalmol Vis Sci. 2012;53(4):1787-91.
23. Geilani AA, Baltomer A, Sahoo S. Bacteriological study on corneal ulcers in the eye outpatient clinics, Great River Eye Hospital, Benghazi. Libyan J Infect Dis. 2007;1(2):121-3.
24. Group HEDS. Predictors of recurrent herpes simplex virus keratitis. Cornea. 2001;20(2):123-128.
25. Prokosch V, et al. Microbiological findings and predisposing risk factors in corneal ulcers. Graefes Arch Clin Exp Ophthalmol. 2012;250(3):369-74.
26. Keay L, et al. Microbial keratitis predisposing factors and morbidity. Ophthalmology. 2006;113(1):109-16.
27. Bharathi MJ, et al. Analysis of the risk factors predisposing to fungal, bacterial & Acanthamoeba keratitis in south India. Indian J Med Res. 2009. 130(6): p. 749-57.
28. Ng, J., F. Fraunfelder, and K. Winthrop, *Review and Update on the Epidemiology, Clinical Presentation, Diagnosis, and Treatment of Fungal Keratitis.* Current Fungal Infection Reports, 2013. 7(4): p. 293-300.
29. Illingworth CD, Cook SD. Acanthamoeba keratitis. Surv Ophthalmol. 1998;42(6): 493-508.
30. Khatab RA, et al. Evaluation of polymerase chain reaction and culture for the diagnosis of corneal ulcer. Life Sci J. 2012;9(3):746-755.
31. Younis MS, et al. Diagnosis of Acanthamoeba keratitis in clinically suspected cases and its correlation with some risk factors. Egypt J Med Sci. 2013; 34(2):527-540.
32. Cao J, et al. Prevalence of infectious keratitis in central China. BMC Ophthalmol. 2014;14:43.
33. Ibrahim Y, Boase D, Cree I. Incidence of infectious corneal ulcers, portsmouth study, UK. J Clinic Experiment Ophthalmol S. 2012;6:2.
34. Gopinathan U, et al. Review of epidemiological features, microbiological

diagnosis and treatment outcome of microbial keratitis: Experience of over a decade. Indian J Ophthalmol. 2009;57(4): 273-9.

35. Lichtinger A, et al. Shifting trends in bacterial keratitis in Toronto: An 11-year review. Ophthalmology. 2012;119(9): 1785-90.

36. Tewari A, et al. Epidemiological and microbiological profile of infective keratitis in Ahmedabad. Indian J Ophthalmol. 2012; 60(4):267-72.

37. Bhadange Y, et al. Role of liquid culture media in the laboratory diagnosis of microbial keratitis. Am J Ophthalmol. 2013; 156(4):;745-51.

APPENDIX I

Approval consent to undergo medical research

Research Title: Laboratory Diagnosis of Bacterial Keratitis in Alexandria Main University Hospital

Main Researcher Name: Zainab Abdelkader

I / "the undersigned" acknowledge that the purpose and duration of the research has been clarified and notified of its benefits to me and to others. I have also been informed that when necessary, the possibility of a change in the steps of the research for my benefit, and in addition to the lack of complications of the research. I was informed that I had the right to leave the search at any time without any accountability. I have also been informed that the confidentiality of research information will be taken into account.

Signature of the subject:

National ID Number:

Phone number:

Researcher's signature:

APPENDIX II

1. **Patient No.** ☐
2. **Name:** ☐
3. **Age:** ☐ **yrs.**
4. **Sex:** **(1-male, 2-female)**
5. **Occupation:**
6. **Date of examination:**
7. **Address:**
8. **Telephone No. :**
9. **Socio-economic status:** ☐
   1. **Urban**
   2. **Rural**
10. **Complaints:**

	Right eye	Left eye	Duration
1. Pain (1- Yes, 2- No)	☐	☐	☐
2. Photophobia (1- Yes, 2- No)	☐	☐	☐
3. Watering (1- Yes, 2- No)	☐	☐	☐
4. Redness (1- Yes, 2- No)	☐	☐	☐
5. Discharge (1- Yes, 2- No)	☐	☐	☐

11. **Previous History of Applications of (1- Yes , 2- No)**
    1. Antibiotic ☐
    2. Atibiotic + Steroid ☐
    3. Cycloplegic ☐
12. **History of Risk Factors (1- Yes , 2- No)**
    1. Trauma ☐
    2. Contact lens wear ☐
    3. Diabetes ☐
    4. Dacrocystitis ☐
    5. Immune suppressor ☐
    6. Recurrence ☐
13. **Medical History:** ☐

    (1- Diabetes, 2- Hypertension, 3- Both, 4- Any Other Medical Disorder, 5- None)
    If 4 Specify:

14. Personal History: ☐

(1- Smoking, 2- None)

15. Occular Examination:

	Right Eye	**Left Eye**
Conjunctiva		
Normal (1= yes; 2= no)	☐	☐
Conjunctival congestion (1=yes; 2=no)	☐	☐
Cornea (1- normal, 2- abnormal)	☐	☐
▪ **Keratitis**	☐	☐
▪ **Corneal ulcer**		
a. Location (1 = Central; 2= Peripheral)	☐	☐
b. Shape		
c. Size		
d. Depth (1=Superficial;2=Mild Stromal; 3=Deep Stromal)	☐	☐
e. Perforation (1=yes; 2=no)	☐	☐
f. Hypopyn (1=yes; 2=no)	☐	☐

16. Ocular Investigations:

A. Corneal scraping

a. Stain (1=positive, 2= negative)

- Gram ☐
- KOH wet mount ☐

b. Culture (1=positive, 2= negative) ☐

- Blood Agar ☐
- Chocolate Agar ☐
- Sabouraud's Dextrose Agar ☐
- Brain Heart Infusion ☐

c. Antibiotic sensitivity test

APPENDIX III

Corneal Scraping Examination, Culture & Sensitivity

Patient #:

If gram positive organisms:

	Test	Principle / Procedure	Result
		Day One	
Day 1	1. Direct Microscopic examination	• Swab of cornea on two slides.	
	2. Culture ✓ On Blood agar (Solid enriched media)	• Culture on blood agar from corneal sample using Komura spatula. • Streak the agar in a C-shaped manner, streaking without burning. • Incubate at 37 ºC 24 hrs. • Incubate at 37 ºC in CO_2 24 hrs.	Day 2 Colonies: Size: Shape:
	✓ On Chocolate agar (non-selective, enriched growth medium) ✓ On Sabaraud dextrose agar (SDA)	• Method: Streak the agar in a C-shaped manner, streaking without burning. • Incubate at 20-25 ºC for 5 days.	
	3. Culture on Brain Heart Infusion Broth (BHIB)	• It is a liquid medium rich in nutrients. • Incubate at 37 ºC 24 hrs.	
		Day Two	
Day 2	1. Indirect Microscopic examination	• A loop full from BHIB or an isolated colony from the blood/chocolate agar.	
	2. Culture - On Blood agar (Solid enriched media)	• Culture on blood agar from Brain heart infusion broth. • Method: primary inoculum, the next inoculum with burning loops, end with tail.	Day 3
	- On Chocolate agar (non-selective, enriched growth medium)	• Incubate at 37 ºC 24 hrs. • Culture on chocolate agar from Brain heart infusion broth.	
	- On Sabaraud dextrose agar (SDA)	• Incubate at 37 ºC 24 hrs. • Method: primary inoculum, the next inoculum with burning loops, end with tail • Incubate at 20-25 ºC for 5 days.	
		Day Three	
Day 3	1. Indirect Microscopic examination	• Isolated Colony from cultured plate.	
	2. Catalase test	• Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; H_2O_2.	
	3. Coagulase test	❖ ***Slide Coagulase Test Procedure(done to detect bound coagulase or clumping factor)*** ❖ ***Tube Coagulase Test Procedure*** *(done to detect free coagulase)*	

		• Incubate tube at 35°C in ambient air for 4 hours.	
	4. Culture on Mannitol salt agar (If suspect staphylococcus spp.)	• Incubate at 37 ºC 24 hrs.	Day 4
	5. Novobiocin Susceptibility	• Novobiocin is an amino-coumarin antibiotic which can be used to differentiate *S. aureus* from some CoNS.	Day 4

If Gram negative organisms:

		Day Two	
Day 2	1. Microscopic examination	• Isolated Colony from cultured plate/ BHIB, Heat fix. • Gram stain; Then examine by oil immersion lens (100x).	
	2. Culture ✓ On Blood agar (Solid enriched media) ✓ On Chocolate agar (non-selective, enriched growth medium) ✓ On MacConkey's agar (Selective & Differential media) ✓ On Nutrient agar ✓ On Cetrimide agar (Selective & Differential media)[If suspect pseudomonas spp.] ✓ On Sabaraud dextrose agar (SDA)	• Culture on blood agar from Brain heart infusion broth. • Streak the agar with burning. • Incubate at 37 ºC 24 hrs. • Method: primary inoculum, the next inoculum with burning loops, end with tail • Incubate at 37 ºC in CO_2 24 hrs. • Contain inhibitor substances (bile salts, crystal violet) • PH indicator Neutral red (red in acid) • Fermentable sugar is Lactose. • Method: primary inoculum, the next inoculum with burning loops, end with tail • Incubate at 37 ºC 24 hrs. • Nutrient agar is a general purpose medium supporting growth of a wide range of non-fastidious organisms. • Plates are usually inoculated by streak or spread method from non-selective medium or directly from the specimen. Incubate the plates at 35- • 37°C for up to 48 hours. • Method: primary inoculum, the next inoculum with burning loops, end with tail. • Incubate at 20-25 ºC for 5 days.	Day 3 Day 4 -5 Day 5-7
	3. Triple Sugar Agar test (TSA) Done on gram negative rods only (enteric pathogens) ✓ Inoculate by: Stab + Streak the slant)	Test the ability of the organism to : 1. Ferment glucose (0.1 %-constitutive enzyme); 2. Utilize lactose – sucrose (1% each - inducible enzymes). 3. Anaerobic respiratory process that use Sulfur as final electron acceptor to produce hydrogen sulfide (Black	Day 3

	✓ Incubate at 37 ºC 24 hrs.	precipitation). 4. Protein [Aerobic process –upper slant if deaminate become red color]. 5. Indicator: phenol red (yellow in acid). 6. Sulfur source: a) Organic amino acid. b) Inorganic Ferrous sulfate.	
	4. Oxidase test	• This test depends on the presence of cytochrome oxidase in bacteria.	
	5. Motility test	• Non biochemical test	
	6. IMViC a) Indole	• Ability of the organism to spit Indole form tryptophan amino acid by tryptophanase enzyme in tryptophane broth. ✓ Incubate at 37 ºC 24 hrs. ✓ Add 0.1 xylol (shake), add Kovac's reagent.	Day 3
	b) Methyl Red	• Test mixed acid producers. • The bacteria maintain stable acid end products from glucose fermentation (large amount of acid from glucose fermentation that overcomes the buffering action. ✓ Inoculate buffered glucose broth. ✓ Incubate at 37 ºC 2-5 days. ✓ Add Methyl red reagent & shake.	Day 4
	c) VP	Test butylene glycol producers. Test the ability of bacteria to produce NEUTRAL end products from fermentation of glucose. ✓ Inoculate buffered glucose broth. ✓ Incubate at 37 ºC 2-5 days. ✓ Add VP reagent – wait 15 min with open cap [Don't shake]	Day 4
	d) Citrate	• Test the ability of the organism to utilize Citrate as sole source of carbon & energy by citritase enzyme. • Streak the slant of simmon citrate agar by inoculated loop. • Incubate at 37 ºC 24 hrs.	Day 3
	7. Urease hydrolysis test	• Test the ability of the organism to hydrolyze urea by urease enzyme producing alkaline product. • Incubate at 37 ºC 24 hrs.	Day 3
	Day Three		
Day 3	**Antibiogram**		**Day 4**
	Generic name	**Trade name**	
	Penicillin		
	1. Oxacillin (OX)		
	Cephalosporin 2nd		
	2. Cefoxitin (FOX) 2nd		
	3. Ceftriaxone (CRO) 3rd IM	Rociphin, Cefotrix, Cefaxon	
	4. Cefotaxime (CTX) 3rd	Claforan	
	5. Ceftazidime (CAZ) 3rd	Fortum	

Glycopeptide		
6. **Vancomycin (VA)**		
Aminoglycoside		
7. Gentamycin (CN)	Apigent , genoptic , Cidomycin	
8. Tobramycin (TOB)	Tobralex, Tobral, Tobrin	
9. Amikacin (AK)		
10. Neomycin		
Fluoroquinolones		
11. Ciprofloxacin (CIP) 2nd	Ciloxan , Ciprofar , Ciprocin , Cipro	
12. Ofloxacin (OFX) 2nd	Optifox,Oculofox,Oflox,Oflicin,Ofloxin	
13. **Norfoxacin (NOR)** 2nd	OptoQ3	
14. **Lomefoxacin (LOM)** 2nd	Okacon, Orchacin	
15. Levofloxacin (LEV) 3rd	Levaquin	
16. Moxifloxacin (MOX) 4th		
Macrolide		
17. Erythromycin (E)	Erythromycin	
18. Azithromycin (AZM)	Zithromax	
19. Clarithromycin (CLR)		
Tetracyclin		
20. Doxycyclin (DO)	Vibramycin	
21. Tetracyclin (TE)		
Bacteriostatic		
22. Fusidic acid (FD)	Fucithalmic	
23. Chloramphenicol (C)	Isoptofenicol	
24. Polymyxin B (Pb)	Polyfax, Polytrin	
25. Clindamycin (DA)		
Day 4 (In case of MRSE/MRSA)		**Day 5**
Oxacillin Resistance Screening Agar Base (Orsab)	• Oxacillin Resistance Screening Agar Base is a nutritious and selective medium containing peptones for growth, a high salt concentration and lithium chloride to suppress non-*staphylococcal* growth with mannitol and aniline blue for the detection of mannitol fermentation.	

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21528